

## Environmental Tobacco Smoke, Genetic Susceptibility, and Risk of Lung Cancer in Never-Smoking Women

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**Background:** Exposure to environmental tobacco smoke (ETS) is considered to be a major lung cancer risk factor for never smokers. We investigated the hypothesis that never-smoking women who are exposed to ETS and develop lung cancer are a genetically susceptible population. **Methods:** Archival tumor tissues were analyzed from 106 never-smoking women enrolled in a case-control study of ETS (and other personal and environmental factors) and lung cancer risk. We analyzed germline polymorphisms in genes that have been associated with cancer susceptibility and whose products activate (cytochrome P450 1A1 [CYP1A1]) and detoxify (glutathione S-transferases M1 [GSTM1] and T1 [GSTT1]) chemical carcinogens found in tobacco smoke. **Results:** When compared with never smokers who had no ETS exposure and developed lung cancer ( $n = 55$ ), never smokers with exposure to ETS who developed lung cancer ( $n = 51$ ) were more likely to be deficient in GSTM1 activity (i.e., were GSTM1 null) because of a genetic polymorphism in the GSTM1 gene (odds ratio = 2.6; 95% confidence interval = 1.1–6.1). A statistically significant rising trend in risk occurred with increasing ETS exposure (two-sided  $P = .02$ ), reaching a more than sixfold excess risk in those exposed to 55 pack-years of ETS (ETS pack-year = ETS produced by an active smoker, within a confined space such as a room, who smokes one pack of cigarettes a day for a year). No evidence was found of associations between GSTT1 deficiency or the CYP1A1 valine variant

and lung cancer risk due to ETS exposure. **Conclusions:** A common genetic polymorphism divides the population of never smokers into two groups of approximately equal size, one (homozygous carriers of the GSTM1 null allele) that has a statistically significant greater risk of lung cancer from ETS than the other (heterozygous or homozygous carriers of the wild-type GSTM1 allele). [J Natl Cancer Inst 1999;91:2009–14]

Although active smoking accounts for 90% of U.S. lung cancer deaths, lung cancer in lifelong never smokers accounted for 11 000 U.S. deaths in 1995 (1). A complex mixture of carcinogenic exposures, socioeconomic factors, diet, and genetics has obscured specific etiologies, but the major causes are considered to be environmental tobacco smoke (ETS) (2), radon (1), diet (3,4), non-neoplastic lung disease (5), and family history of lung cancer (6,7). Current data indicate that U.S. lung cancer risks are increased approximately 1.6-fold by ETS [reviewed in (8)], and a recent, 10-year European study of 2000 adults (9) found a small, but definitely, elevated risk.

Several lung cancer susceptibility genes have been proposed, and enzymes activating or detoxifying chemical carcinogens have been investigated [reviewed in (10,11)]. To date, several cytochrome P450 pathway enzymes that activate chemical carcinogens and several glutathione S-transferase (GST) enzymes that detoxify chemical carcinogens have been associated with lung cancer susceptibility (12–16). The human GSTs are phase II detoxification enzymes encoded by four classes of polymorphic genes: alpha, mu, pi, and theta [reviewed in (17–19)]. All of these enzymes detoxify carcinogens and reactive oxygen species by conjugating them to glutathione, and alterations in the mu and theta class genes have been linked to tobacco-associated lung cancers. The mu class includes at least five genetic variants, and GSTM1 is notable for a “null” allele inactivated by a deletion of DNA coding sequences (20). Loss of GSTM1 enzymatic activity due to the homozygous null genotype occurs in about 50% of white populations of Europe and North America [reviewed in

(21–23)], and it has been linked to increased risks of tobacco-associated cancers of the lung (12), head and neck (24,25), larynx (26), and bladder (27–30). Compared with men, women with the GSTM1 null genotype may have greater risks of tobacco-associated cancers (31). A meta-analysis of 1593 patients with lung cancer and 2135 control subjects concluded that GSTM1 deficiency confers an additional 40% risk of lung cancer to the individual cigarette smoker (odds ratio [OR] = 1.4; 95% confidence interval [CI] = 1.2–1.6) and accounts for 17% of all lung cancers because of its high prevalence (12).

The theta class of GSTs contains two isoenzymes including GSTT1 (32), which has an inactivating homozygous deletion polymorphism that occurs in 11%–18% of whites (21). A functional deficiency of this enzyme activity (32) was associated with increased risks of smoking-associated laryngeal and bladder cancers (21), and combined deficiency of both GSTT1 and GSTM1 produced a substantial susceptibility to lung cancer in Finnish (16), American (33), and French (34) populations. The cytochrome P450 1A1 (CYP1A1) enzyme activates carcinogenic polycyclic aromatic hydrocarbons including the benzo[a]pyrene component of tobacco smoke (35), and a polymorphic valine allele in exon 7 increases both enzymatic activity and lung cancer risks in Japanese smokers [reviewed in (36)]. Furthermore, there is evidence for a gene-gene interaction between the variant (i.e., “mutant”) CYP1A1 allele and homozygous deletion of GSTM1 to produce a more than additive risk of lung cancer in

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most Japanese and some white smokers [reviewed in (21)].

We extended the classical epidemiologic observations on ETS and lung cancer by conducting a molecular epidemiologic study of gene-environment interactions promoting lung cancer in never-smoking women. Our study was designed specifically to investigate ETS and lung cancer with the use of a population-based series of never-smoking case patients, and it employed telephone and in-person structured interviews to obtain information about multiple lung cancer risk factors, including ETS exposure, age, and intake of vegetables and animal fat (2,4,37). We examined dose-response relationships between ETS and lung cancer risk among women with functional genetic polymorphism for enzymes that activate (i.e., CYP1A1) and detoxify (i.e., GSTM1 and GSTT1) tobacco smoke carcinogens.

## METHODS

**Study design.** Archival, paraffin-embedded lung cancer tissues from therapeutic resections or diagnostic biopsies were collected from white women in Missouri who participated in a population-based, case-control study of lung cancer in never smokers and long-term ex-smokers (2,4,6,38-40). The original epidemiologic study was designed to measure the risks of lung cancer conferred by ETS, home radon, diet, family history of cancer, occupational exposure to known causes of lung cancer, and non-neoplastic lung disease. The decision to use a case-only study design to assess gene-environment interaction did not hamper our analysis because this approach has been previously shown to yield the same estimator of interaction effect as do studies that collect complete data on cases and controls (41,42). Missouri was chosen because of its stable population and its population-based cancer registry—which includes smoking status information—and the series was limited to whites because of the small numbers of other racial and ethnic groups in the state. Lung cancer patients were identified from the Missouri Cancer Registry for a 5-year period and included 432 lifetime never smokers and 186 long-term ex-smokers (2).

**Exposure dosimetry for ETS.** ETS exposure was quantified by telephone interviews determining the source (e.g., parent or spouse), intensity, and duration of exposure during childhood and adulthood (2). One ETS pack-year is the exposure, within a confined space such as a room, to ETS produced by an active smoker consuming one pack of 20 cigarettes each day for a year.

**Sample collection.** Tissue samples were requested for all 618 lung cancers from never smokers and ex-smokers in the epidemiologic study (2,4,6,38-40); request letters were sent to hospitals where resections or diagnostic biopsies were performed. Archival, formalin-fixed, paraffin-embedded, tumor tissue samples were provided for 132 (21% of requested) patients, including 11 ex-

smokers (who were excluded from further analyses) and 121 never smokers. Composite histologies, average ages, and educational levels for the 106 never smokers whose tissues yielded DNA and data on GSTM1 status are compared with results for the entire series of 618 never smokers and ex-smokers to show that the subset analyzed in this report is comparable to the full series (Table 1).

### Genetic analyses of cancer susceptibility genes.

Tumor and nontumor tissues were microdissected from histologic sections with the use of protocols to minimize polymerase chain reaction (PCR) contamination (43), and genomic DNA was isolated from nontumor tissues with the use of standard procedures (44). The CYP1A1 exon 7 isoleucine-to-valine substitution and the GSTM1 deletion were determined by multiplex PCR amplification and gel electrophoresis as previously reported (45). The GSTT1 deletion was also analyzed by multiplex PCR with the use of an allelic discrimination system (7700 Sequence Detector ["TaqMan"]; Applied Biosystems Inc., Norwalk, CT). PCR primers and fluorescent-labeled probes were employed to discriminate present and absent alleles by differential fluorescent signals for GSTT1 and  $\beta$ -actin (GSTT1 Kit; BioServe Biotechnology Inc., Laurel, MD). This assay was validated by duplicate analysis and by polymorphic mendelian inheritance patterns in seven human family cell lines (data not shown; National Institute of General Medical Sciences Human Genetic Mutant Cell Repository, Coriell Institute for Medical Research, Camden, NJ). Although neither assay could distinguish between one or two alleles of GSTM1 or GSTT1, homozygous deletions were determined unequivocally.

**Statistical analysis.** ORs and 95% CIs were calculated by multiple logistic regression (41), and all comparisons were adjusted for age and other significant factors as defined in previous studies (2,4,6,38-40) (i.e., history of non-neoplastic lung disease, radon exposure, and intake of saturated fat and vegetables). All statistically significant associations were retested with the use of exact methods (46), but adjustments for confounding variables with the use

of exact methods could not be performed by commercially available software in this dataset. All *P* values were calculated as two-sided statistics, which were considered to be significant for *P* < .05. The linearity of trends in risk according to levels of exposure to ETS was evaluated with a score test, which is equivalent to the Mantel extension test for linear trend (41). All comparisons were adjusted for age and other significant factors as defined in previous studies (2,4,6,38-40) (i.e., history of non-neoplastic lung disease, radon exposure, and intake of saturated fat and vegetables). One can estimate multiplicative gene-environment interactions in logistic models with data from case patients alone with even greater precision than from both case patients and control subjects, provided that the environmental factor and the genotype are statistically independent in the population and the disease is rare (42,47). It is unlikely that GSTM1 status is associated with adult ETS exposure, particularly among lifetime never smokers.

## RESULTS

### Descriptive, Laboratory, and Statistical Analyses of the Missouri Women's Lung Cancer Study

The current series of 106 never-smoking, white women with lung cancer is representative of the 618 cases in the original epidemiologic study as shown in Table 1 (2,4,6,38-40). Compared with the complete population-based sample, the case patients reported here were identical in race and sex and similar in mean age at diagnosis (69.8 years versus 71.5 years) and education level. Similarly, the tumors in this study were comparable in proportion of histologic subtypes to those in the original study, with slight

**Table 1.** Comparisons of the current case patient series to the original population-based case patient series\*

Parameter	Current series	Original series
No. of patients	106	618
Age range, y (mean)	41-84 (69.8)	30-84 (71.5)
Histology, No. (%)†		
Adenocarcinoma	75 (71)	292 (62)
Bronchoalveolar	8 (8)	19 (4)
Squamous cell carcinoma	4 (4)	27 (6)
Small-cell lung cancer	2 (2)	12 (3)
Other/mixed	17 (16)	118 (25)
Smoking history, No. (%)		
Never smoker	106 (100)	432 (70)
Ex-smoker	0	186 (30)
Education, No. (%)		
<12 y	36 (34)	240 (39)
12 y	43 (41)	228 (37)
>12 y	25 (24)	121 (20)
Unknown	2 (2)	29 (5)

\*Descriptions of the original, population-based, case patient series have been published (2,4,6,38-40).

†Histologic materials from 468 case patients were available for morphologic confirmation by an expert panel of pathologists (6).

excesses of adenocarcinomas (71% versus 62%) and bronchoalveolar carcinomas (8% versus 4%) and slight deficits in squamous cell carcinomas (4% versus 6%), small-cell lung cancers (2% versus 3%), and other/mixed histologies (16% versus 25%) (Table 1). The only substantial difference was in the proportion of ex-smokers, who accounted for 30% of the original population but who were specifically excluded from the current study.

The GSTM1 genotypes were determined for the 106 never smokers as 60% absent (i.e., homozygous for the null allele) and 40% present (i.e., with one or two functional alleles present; Table 2). The slight excess of null alleles is typical for the lung cancer series among the white population (12), and the average age at diagnosis of the homozygous null group was slightly, but not statistically significantly, greater than the heterozygous and homozygous wild-type genotypes, 70.0 years versus 69.4 years. Data on the GSTT1 genotypes were available for 65 patients, with 18% absent (i.e., homozygous null) and 82% present (i.e., having one or two functional alleles; Table 3); similar frequencies for white populations have been reported by multiple investigators [reviewed in (21)]. There were 95 (91%) case patients with two wild-type CYP1A1 alleles and nine (9%) with one or two mutant alleles (Table 3); similar frequencies have been reported for other white populations (36).

**Table 2.** Case patient-only analysis of environmental tobacco smoke (ETS) exposure: association with glutathione *S*-transferase (GSTM1) genotype

ETS exposure*		GSTM1 genotype†		
Quartile	Pack-years	Absent	Present	OR (95% CI)‡
1	0	27	28	1
2	0–20	13	6	2.1 (0.7–6.8)
3	21–55	13	6	1.9 (0.6–6.0)
4	>55	11	2	6.5 (1.2–35.0)§
	Any ETS	37	14	2.6 (1.1–6.1)§
	Trend test			<i>P</i> = .02§

\*ETS exposure is shown as quartiles for GSTM1 analysis, where 0 pack-years of exposure was defined as the first quartile, and the second through fourth quartiles were constructed from approximately equal divisions of the remaining case patients.

†Absent means both alleles are null or deleted. Present means one or two intact alleles are present.

‡OR (95% CI) = odds ratio (95% confidence interval) adjusted for age, radon exposure, saturated fat intake, and vegetable intake.

§Exact inference reproduced the sixfold elevation of risk above 55 pack-years of exposure (OR = 5.6; 95% CI = 1.1–5.63, with *P* = .01 for the trend test), and the categorical risk associated with any ETS exposure was unchanged (OR = 2.7; 95% CI = 1.1–6.7). Note that commercially available software for exact computations could not adjust for age, radon exposure, saturated fat intake, and vegetable intake in this dataset.

||Categorical trend test is expressed as a two-sided *P* value, which is considered statistically significant for *P* < .05.

**Table 3.** Case patient-only analysis of environment tobacco smoke (ETS) exposure: association with glutathione *S*-transferase T1 (GSTT1) and cytochrome P450 1A1 (CYP1A1) genotypes

ETS exposure*		GSTT1 genotype†			CYP1A1 genotype		
Quartile	Pack-years	Absent	Present	OR (95%)‡	MM/MM§	WW§	OR (95% CI)‡
1	0	7	26	1	5	49	1
2	0–20	3	9	1.1 (0.2–6.3)	2	15	1.4 (0.2–9.4)
3	21–55	1	12	0.3 (0.0–2.8)	1	19	0.3 (0.0–3.8)
4	>55	1	6	0.6 (0.0–7.3)	1	12	0.7 (0.1–7.8)
	Any ETS	5	27	0.6 (0.1–2.4)	4	46	0.7 (0.2–3.3)
	Trend test			<i>P</i> = .29			<i>P</i> = .50

\*ETS exposure is shown as quartiles for GSTM1 analysis, where 0 pack-years of exposure was defined as the first quartile, and the second through fourth quartiles were constructed from approximately equal divisions of the remaining case patients.

†Absent means both alleles are null or deleted. Present means one or two intact alleles are present.

‡OR (95% CI) = odds ratio (95% confidence interval) adjusted for age, radon exposure, saturated fat intake, and vegetable intake.

§MM = val/val exon 7 polymorphism; MM = ile/val genotype; WW = ile/ile genotype.

||Categorical trend test is expressed as a two-sided *P* value, which is considered statistically significant for *P* < .05.

### Lung Cancer Risks for Never Smokers Determined by Gene–Environment Interactions

**Association of the GSTM1 homozygous null genotype with substantial lung cancer risk at high levels of ETS exposure.** Within the group of 106 never smokers, there was a surplus of the GSTM1 homozygous null genotype (i.e., 60%); however, in the absence of ETS exposure, the GSTM1 null genotype was a slight minority (27 versus 28 case patients; Table 2). Excess GSTM1 null alleles occurred in the presence of ETS exposure and outnumbered the GSTM1-positive case patients by 2:1 in the second and third quartiles of ETS expo-

sure and by 6:1 in the fourth quartile. The first quartile was defined as absence of exposure to ETS, and the other quartiles were approximately equal groups of the remaining patients; the lower boundary for the fourth quartile was drawn at 55 pack-years of exposure because it represented a natural clustering of 13 case patients at the top of the exposure scale. Case patient-only analysis, adjusted for confounding variables, determined that exposure to more than 55 pack-years of ETS produced a 6.5-fold increased risk of lung cancer for women with the GSTM1 homozygous null genotype (OR = 6.5; 95% CI = 1.2–35.0), and the trend test supported this judgment (*P* = .02; see Table 2). To exclude the possibility that the dataset was too sparse or unbalanced to assure the validity of asymptotic likelihood-based inference, we applied exact methods and found a similar risk estimate (OR = 5.6; 95% CI = 1.1–56.3; trend test *P* = .01), although adjustments for confounding variables including age, radon exposure, saturated fat consumption, and vegetable intake could not be performed with the use of the current software. The categorical comparison of “no ETS” to “any ETS” demonstrated a 2.6-fold increased risk among GSTM1 null genotypes (OR = 2.6; 95% CI = 1.1–6.1) by use of logistic regression with adjustments and a 2.7-fold elevation (OR = 2.7; 95% CI = 1.1–6.7) by use of exact inference without adjustment for confounding variables.

**Lack of GSTT1 and CYP1A1 effects on cancer risks from ETS exposure.** Similar to some, but not all, studies of smoking-associated lung cancer (21), this



case series provides no evidence that GSTT1 deficiency is associated with any lung cancer risk due to ETS exposure ( $P = .29$ , trend test; Table 3), and the GSTT1 homozygous null group is too small to detect a genotypic interaction with GSTM1. Likewise, there is no evidence that the CYP1A1 valine variant in exon 7 is associated with lung cancer risk from ETS exposure ( $P = .50$ , trend test; Table 3), either alone or in concert with the GSTM1 homozygous null genotype.

## DISCUSSION

This is the first investigation to find a dose-response relationship between ETS exposure and increasing lung cancer risk among women with a common genetic deficiency in GSTM1 enzymatic activity. These data indicate that ETS exposure may more than double the risk of lung cancer for nearly half of white women in Western nations. In addition to the overall doubling of risk, there is a highly significant dose-response trend, with ETS exposure ( $P = .02$ ) producing a more than sixfold risk at the highest exposures (OR = 6.5; 95% CI = 1.2–35.0). Although this evidence for a gene-environment interaction is statistically significant, it should be noted that the confidence limits around our estimate of excess risk are wide because of a relatively small sample size. If we were to estimate the interaction OR algebraically by assuming 1) that the main effect of ETS and lung cancer is an OR of 1.6 (8), 2) that ETS does not increase the lung cancer risk among the GSTM1-positive population, and 3) that the prevalence of the GSTM1 homozygous null genotype is 50%, then we would predict an interaction OR between the GSTM1 homozygous null genotype and ETS to be 2.2. Based on our observed estimates of the interaction OR (OR = 2.6), the fraction of lung cancer cases among never-smoking women resulting from this gene-environment interaction would be 32%, while the proportion would be 26% based on our algebraic estimate (OR = 2.2). However, for the half of the population of never-smoking women with the GSTM1 null polymorphism, ETS exposure is responsible for between 42% and 49% of the lung cancer cases. The risks for women of other races and men may be similar, although direct evidence is not yet available. In addition to ETS, it has been estimated that 17% of lung cancers from smokers (12) and 17% of bladder cancers from smokers (30)

may be attributed to deficiency of GSTM1 enzymatic activity. These latter appraisals are based on case-control data from multiple studies using the two cancer sites for which consistent GSTM1 deficiency associations have been reported. These approximations suggest that the GSTM1 homozygous null genotype is a major determinant of lung cancer susceptibility, possibly because its substrate specificities may differ from those of other GST classes (48).

This observation may have escaped prior notice because lung cancer series typically enroll active smokers, and it is possible that GSTM1 deficiency is most clinically significant at low carcinogen doses (49,50), such as with ETS exposure, because other detoxification pathways may be overloaded by active smoking (15,51,52). In addition, this study was designed specifically to measure the risks of ETS exposure (2), so never smokers were chosen as the primary study population (6), and the survey instruments were selected to optimize exposure assessment (53,54) and to minimize misclassification of never smokers (39,55–57). Recent results (31) also indicate that women with a GSTM1 homozygous null genotype may be at greater risk of developing lung cancer when compared with men who carry this “at risk” genotype. Additional studies are needed to confirm the risks of the ETS-GSTM1 homozygous null genotype interaction in white women and to expand the observation to other races and both sexes.

Tobacco smoke has many substrates for GSTM1, GSTT1, and CYP1A1, and individuals with multiple susceptibility alleles at these and other loci should have a greater risk of developing smoking-related lung cancer than those who carry only one such allele. One of the best known genetic interactions is the combination of GSTM1 homozygous null genotype and the valine allele in exon 7 of CYP1A1, which may produce 20-fold risks in Japanese smokers [reviewed in (21)]. However, similar to other series of white smokers (36), the CYP1A1 valine allele did not enhance the risk of ETS exposure among these never smokers, either alone or in combination with homozygous GSTM1 null genotype. This is possibly a statistical effect because the CYP1A1 valine allele is common among Japanese but relatively uncommon in whites; however, it is a good example of the ethnic and/or environmental varia-

tions that must be considered in investigations of causal factors. In addition, genetic interactions between null alleles of GSTM1 and GSTT1 have been suggested (33,34), but the low frequency of homozygous GSTT1 null alleles (i.e., 17%) does not permit such an assessment in our relatively small dataset. Although the absence of GSTT1 enzymatic activity has been sometimes associated with increased lung cancer risk in active smokers (21), these data do not support an interaction with ETS.

In short, we find that the GSTM1 homozygous null genotype is associated with a statistically significant lung cancer risk in never-smoking women exposed to high levels of ETS, which suggests that the observed excess lung cancer risk among never-smoking women results from cancers in two distinct groups: one that is genetically at high risk and one that is genetically at lower risk of lung cancer from exposure to ETS. Additional studies are needed to confirm these observations and to investigate the contributions of other detoxification pathways to lung cancer risk.

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## NOTES

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We thank Dr. Ross C. Brownson for valuable assistance in accessing tissue samples, Dr. Jay H. Lubin for helpful discussions, Ms. Tuulikki Kärnä and Mr. Michael Bartel for laboratory assistance, and Ms. Dorothea Dudek for editorial assistance.

Received February 11, 1999; revised September 7, 1999; accepted September 28, 1999.